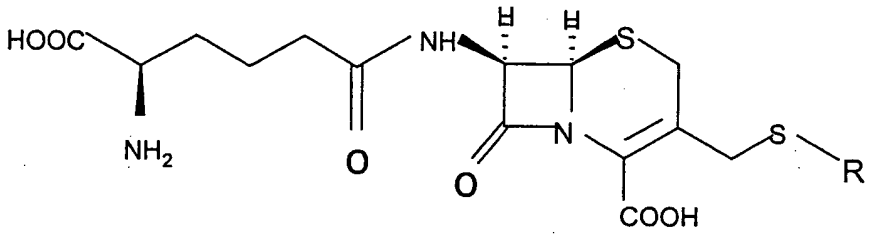
Claims

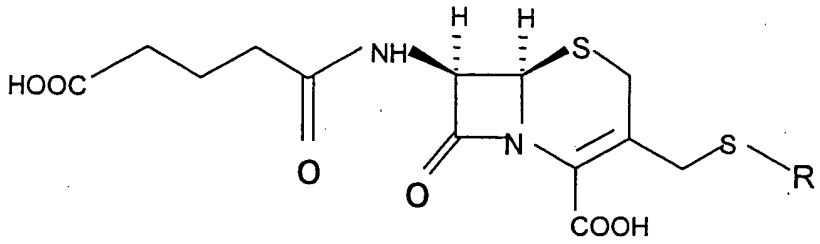
1. An enzymatic process for preparing 3-thiolated 7-aminocephalosporanic acid derivatives comprising the steps:-

enzymatically converting a 3-thiolated cephalosporin C of the formula I:



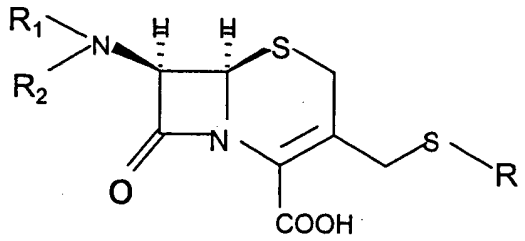
(I)

to form a 3-thiolated –glutaryl-7-ACA of the formula II



(II)

and enzymatically converting a compound of formula II to form a 3-thiolated-7-ACA of the formula III



(III)

wherein R is a heterocyclic group comprising at least one nitrogen atom and R_1 and R_2 are both hydrogen atoms or one of them is a hydrogen atom and the other is an acyl donor.

2. A process as claimed in claim 1 wherein the 3-thiolated cephalosporin C of formula I is converted into a 3-thiolated-glutaryl-7-ACA of the formula II by:-

reacting a compound of formula I with immobilised D-Amino acid oxidase in the presence of molecular oxygen;

separating the supported enzyme from the aqueous reaction mixture; and

adding hydrogen peroxide to convert the 3-thiolated-α-ketoadipyl cephalosporanic acid into a compound of formula II.

3. A process as claimed in claim 2 wherein the compound of formula I is reacted with immobilised D-Amino acid oxidase at a pressure of about 2 bar absolute, a pH of from 6.0 to 8.0, and a temperature of from 20°C to 30°C for a period of from 0.5 to 3 hours.

4. A process as claimed in claim 2 including the step of washing the supported enzyme with a concentrated salt solution and adding hydrogen peroxide preferably in an amount equivalent to 30 to 50 ppm to the solution thus formed.

5. A process as claimed in claim 1 comprising the step of eliminating excess hydrogen peroxide from the solution, preferably by adding a catalyst to the solution.

6. A process as claimed in claim 5 wherein the excess hydrogen peroxide is removed by adding catalase to the solution.

7. A process as claimed in claim 1 wherein a compound of formula II is converted into a compound of formula III by contacting a compound of formula II with immobilised glutaryl-7-ACA acylase.

8. A process as claimed in claim 7 wherein the reaction to form a compound of formula III from a compound of formula II is carried out at ambient pressure, at a pH of from 6.0 to 8.5 and at a temperature of from 20°C to 35°C, for a period of from 0.5 to 3 hours under an inert atmosphere.

9. A process as claimed in claim 7 wherein the compound of formula III is precipitated by acidifying the reaction medium and the precipitate thus formed is subsequently washed and dried.

10. A process as claimed in claim 1 wherein the enzymes are immobilised using a suitable cross-linker agent in a suitable solid support.

11. A process as claimed in claim 10 wherein the enzymes are in the form of crystals of a size suitable for use as a biocatalyst.

12. A process as claimed in claim 1 wherein the enzymatic processes are carried out while maintaining the enzyme in dispersion in an aqueous substrate solution.

13. A process as claimed in claim 1 wherein the or each enzymatic process is carried out in a column.

14. A process as claimed in claim 1 including the step of recovering the enzyme for reuse.

15. A process as claimed in claim 1 wherein crystallisation of a compound of formula III is carried out at an acidic pH.

16. A process as claimed in claim 1 wherein the enzymatic conversion of a 3 thiolated cephalosporin C of the formula I to form a 3 thiolated-7-ACA of the formula III is carried out in one pot.

17. A process as claimed in claim 1 wherein R is a heterocyclic group comprising at least one nitrogen atom and optionally a sulphur or oxygen atom.

18. A process as claimed in claim 1 wherein R is a heterocyclic group selected from any one or more of the group comprising thienyl, diazolyl, tetrazolyl, thiazolyl, triazinyl, oxazolyl, oxadiazolyl, pyridyl, pirimidinyl, benzo thiazolyl, benzimidazolyl, benzoxazolyl, or any derivative thereof,

preferably 5-methyl-1,3.4-thiadiazol-2-yl, 1-methyl-1H-tetrazol-5-yl or 1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl.

19. A process as claimed in claim 1, wherein compounds of formula I are in a solid form or in the form of a non-toxic salt thereof.

20. A 3-thiolated-7-ACA of the formula III whenever prepared by a process as claimed in claim 1.

21. A process for the preparation of cephalosporin C antibiotics and derivatives thereof comprising forming a compound of formula III as defined in claim 1 and subsequent enzymation.

22. A process as claimed in claim 21 wherein the antibiotic is cefazolin, cefazedone, cefoperazone, cefamandol, cefatriazine, cefotiam or ceftriaxone.

23. An enzymatic process for preparing 3-thiolated 7-aminocephalosporanic acid derivatives as claimed in claim 1 comprising the steps:-

reacting cephalosporin C with a thiol compound of the general Formula IV

R-SH (IV)

wherein R is a heterocyclic group comprising at least one nitrogen atom,

to form a compound of formula I

and, after formation of the compound of formula I removing excess thiol of Formula IV.

24. A process as claimed in claim 23 wherein the excess thiol is removed by adsorption on an anion exchange resin.

25. A process as claimed in claim 24 wherein the anion exchange resin is a microporous resin having a cross-linked acrylic copolymer structure.

26. A process as claimed in claim 25 wherein the anion exchange resin comprises an 8% cross-linking containing functional thialkyl benzyl ammonium group.

27. A process as claimed in claim 25 wherein the resin is in the chloride, hydroxy, phosphate or acetate cycle.

28. A process as claimed in claim 23 wherein the excess thiol is removed by crystallisation.

29. A process as claimed in claim 28 wherein crystallisation is carried out at an acidic pH.

30. A process as claimed in claim 23 wherein the excess thiol is removed by crystallisation followed by adsorption on an anion exchange resin.

31. A process as claimed in claim 23 wherein the cephalosporin C is in an aqueous medium.

32. A process as claimed in claim 23 wherein the cephalosporin C is in the form of a concentrated cephalosporin C solution.

33. A process as claimed in claim 23 wherein the reaction is carried out at a pH of between 5.5 and 8.0, at a temperature of from 60°C to 80°C, for a period of from 1 to 12 hours.

34. A process as claimed in claim 33 wherein the reaction is carried out at a pH of approximately 6.0 and at a temperature of approximately 65°C.

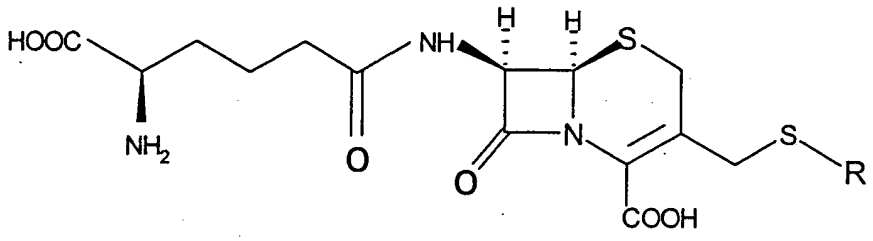
35. A process as claimed in claim 23 wherein the thiol compound is present in an amount of between 1 and 5 mol/mol of cephalosporin C.

36. A process as claimed in claim 23 wherein R is a heterocyclic group comprising at least one nitrogen atom and optionally a sulphur or oxygen atom.

37. A process as claimed in claim 23 wherein R is a heterocyclic group selected from any one or more of thienyl, diazolyl, thiazolyl, tetrazolyl, thiadiazolyl, triazinyl, oxazolyl, oxadiazolyl, pyridyl, pirimidinyl, benzothiazolyl, benzimidazolyl, benzoxazolyl, or any derivative thereof, preferably 5-methyl-1,3,4-thiadiazol-2-yl, 1-methyl-tetrazol-5-yl or 1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl.

38. A process as claimed in claim 23 wherein compounds of Formula I are in a solid form or in the form of a non-toxic salt thereof.

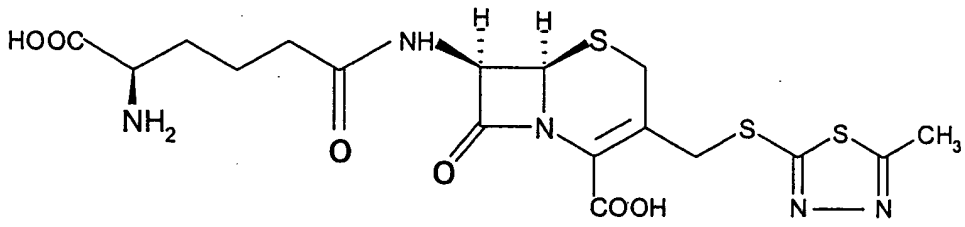
39. A compound of formula:-



wherein R is a heterocyclic group comprising at least one nitrogen atom,

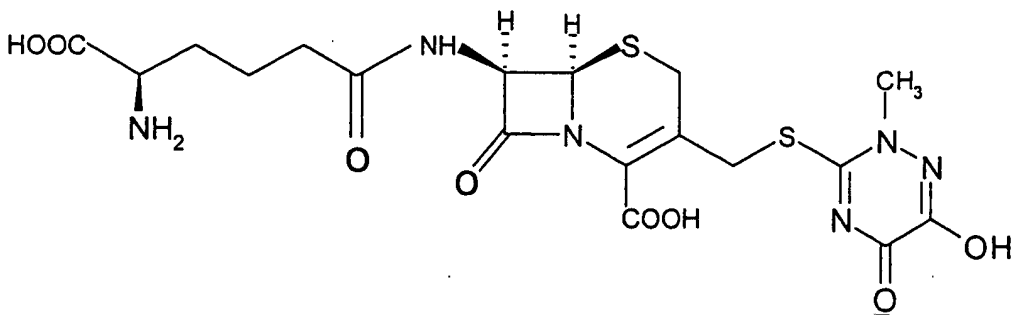
obtained by a process as claimed in any preceding claim.

40. A compound of the formula:-



wherein in formula I R is 5-methyl-1,3,4-thiadiazol-2-yl.

41. A compound of the formula:-



wherein in formula I, R is 1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl.

42. A process for the preparation of cephalosporin C antibiotics and derivatives thereof comprising forming a compound of formula I as defined in claim 1 and subsequent enzymation of the compound of formula I.

43. A process as claimed in claim 42 wherein the antibiotic is cefazolin, cefazedone, cefoperazone, cefamandol, cefatriazine, cefotiam and ceftriaxone.